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MECHANISMS OF DISEASE

FRANKLIN H. EPSTEIN, M.D., *Editor*TRANSFORMING GROWTH FACTOR β
IN TISSUE FIBROSISWAYNE A. BORDER, M.D.,
AND NANCY A. NOBLE, PH.D.

PROGRESSIVE fibrosis in the kidney, liver, lung, heart, bone marrow, and skin is both a major cause of suffering and death and an important contributor to the cost of health care. All of this is likely to change in the future. Advances in cell and cytokine biology have brought a new understanding of the molecular events underlying tissue fibrosis. It is becoming clear that fibrogenesis is not a unique pathologic process but is due to excesses in the same biologic events involved in normal tissue repair.¹

A central event in tissue repair is the release of cytokines in response to injury. Several lines of evidence point to transforming growth factor β (TGF- β) as a key cytokine that initiates and terminates tissue repair and whose sustained production underlies the development of tissue fibrosis.² This review will explore the biology of tissue repair and the properties and actions of TGF- β that make it such a potent fibrogenic molecule. Understanding the actions of TGF- β in fibrosis could lead to the development of clinically useful antifibrotic agents.

CYTOKINES IN TISSUE INJURY

Tissue is made up of organized groups of cells attached to an extracellular matrix and surrounded by a network of blood vessels. Tissue homeostasis is maintained by coordinating cell growth and proliferation with the production and turnover of the extracellular matrix. Cells achieve this coordination by constant signaling to themselves (autocrine activity) and each other (paracrine activity) by means of polypeptides called cytokines (also known as growth factors).³ Cytokines differ from conventional hormones in that they act locally, not at a distant site. The action of a cytokine can be positive or negative, depending on the influence of other cytokines and the physiologic state of the target cell and the surrounding extracellular matrix. This variability of cytokine action provides cells and tissues with a range of potential responses to any stimulus.⁴ Cytokines regulate all aspects of tissue

remodeling, whether planned (as in embryogenesis and development) or unplanned (as in carcinogenesis and tissue repair after injury).^{5,6}

TGF- β is a prototypical, multifunctional cytokine that was isolated from platelets and characterized just over 10 years ago.⁷ The name is derived from the observation that TGF- β stimulates normal cells to grow in soft agar as though they had been virally transformed. In mammals the cytokine has three isoforms, TGF- β 1, 2, and 3, whose biologic properties are nearly identical. The TGF- β 1 gene is up-regulated in response to tissue injury, and TGF- β 1 is the isoform most implicated in fibrosis. In this review the generic term TGF- β is used in discussing properties that are probably shared by all three isoforms; the specific isoform is mentioned, however, if it has been identified or used in a particular study.

TGF- β 1, synthesized as a 391-amino-acid precursor molecule, is proteolytically cleaved to yield peptide fragments and a 112-amino-acid subunit. Active TGF- β 1 is a 25-kd dimeric protein composed of two subunits linked by a disulfide bond. TGF- β 1 is secreted in an inactive (latent) form that requires activation before it can exert a biologic effect. The latent form of TGF- β 1 is a high-molecular-weight complex in which TGF- β 1 is noncovalently bound to another dimeric peptide, the latency-associated peptide, which is formed from cleavage fragments of the TGF- β 1 precursor. Latent TGF- β 1 is stored at the cell surface and in the extracellular matrix and is converted to active TGF- β 1 at these sites by an unknown mechanism.⁸

TGF- β binds to at least three membrane proteins, referred to as receptor types I, II, and III, that exist on virtually all cells. The type I and type II receptors are transmembrane serine-threonine kinases that interact with one another and facilitate each other's signaling.⁹ The type III receptor, also called betaglycan, is a membrane-anchored proteoglycan that has no signaling structure but acts to present TGF- β to the other receptors.¹⁰ The effects of TGF- β on the synthesis and deposition of extracellular matrix are mediated by the type I receptor. The effects on cell growth and proliferation are mediated by the type II receptor. The regulation of TGF- β 1 secretion and action involves complex post-transcriptional events, including messenger RNA (mRNA) stabilization, the assembly and activation of the latent TGF- β 1 complex, and the modulation of receptor expression.¹¹

Other cytokines involved with TGF- β 1 in tissue remodeling after injury are platelet-derived growth factor, basic fibroblast growth factor, tumor necrosis factor, and interleukin-1.¹ Each cytokine has distinctive, synergistic roles in tissue repair, as recent studies involving *in vivo* gene transfection, gene disruption ("knockout"), and the administration of cytokines have shown.¹²⁻¹⁵ The dominant effect of platelet-derived growth factor is to stimulate cell proliferation and migration; fibroblast growth factor induces the

From the Division of Nephrology, University of Utah School of Medicine, 50 N. Medical Dr., Salt Lake City, UT 84132, where reprint requests should be addressed to Dr. Border.

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formation of new blood vessels (angiogenesis); and tumor necrosis factor and interleukin-1 promote inflammation, cell migration, and proliferation. TGF- β 1 is unique in its widespread actions that enhance the deposition of extracellular matrix. It also acts as a potent regulator of repair, coordinating or suppressing the actions of other cytokines.^{7,16}

BIOLOGIC ACTIONS OF TGF- β IN TISSUE REPAIR

The healing of a dermal wound, a paradigm for tissue repair in general, is a coordinated sequence of biologic events beginning with platelet-induced hemostasis, followed by an influx of inflammatory cells and fibroblasts, the formation of new extracellular matrix and blood vessels (granulation tissue), and the proliferation of cells to reconstitute the tissue.¹ TGF- β 1 plays an important part in each of these events, which can largely be reproduced in normal tissue by the administration of TGF- β 1.^{16,17} Platelets contain high concentrations of TGF- β 1 and platelet-derived growth factor that are released into the tissue at the site of injury. Inactive (latent) TGF- β 1, bound locally to the extracellular matrix, can also be activated after injury. In femtomolar concentrations TGF- β 1 is strongly chemotactic for neutrophils, T cells, monocytes, and fibroblasts.^{16,18,19} Moving to the site of the injury, these cells become activated as they encounter higher (picomolar) concentrations of TGF- β 1. Monocytes begin secreting fibroblast growth factor, tumor necrosis factor, and interleukin-1, and fibroblasts increase their synthesis of extracellular-matrix proteins.¹⁶ TGF- β 1 also induces both infiltrating cells and

resident cells to produce more of itself. This autoinduction amplifies the biologic effects of TGF- β 1 at the injury site and may have a central role in chronic fibrosis.²⁰

At physiologic concentrations, TGF- β 1 regulates platelet-derived growth factor (in smooth-muscle cells and fibroblasts), fibroblast growth factor (in endothelial cells), and tumor necrosis factor and interleukin-1 (in monocytes) by stimulating or inhibiting their production or modulating their actions to both synchronize and control the repair process.^{21,22} TGF- β 1 also inhibits the functioning of T cells and B cells and their production of tumor necrosis factor and interleukin-1.²³ Neonatal mice in which the *TGF-β1* gene has been inactivated live for several weeks, until the maternal supply of TGF- β 1 is gone, and then die of a systemic autoimmune-like disease in which tissue concentrations of tumor necrosis factor and interleukin-1 are markedly elevated.^{13,14} TGF- β 1 also modulates the cytotoxicity of macrophages by suppressing the production of superoxide and nitric oxide.^{16,24}

Whereas TGF- β 1 can function as either an agonist or an antagonist of cell proliferation and inflammation, it consistently and potently acts on cells to induce the deposition of extracellular matrix.⁷ The accumulation of matrix in tissues is the chief pathologic feature of fibrotic diseases. Extracellular matrix is a dynamic superstructure of self-aggregating macromolecules, including fibronectin, collagens, and proteoglycans, to which cells attach by means of surface receptors called integrins.²⁵ The matrix surrounding cells is continually degraded by proteases. Figure 1 illustrates how

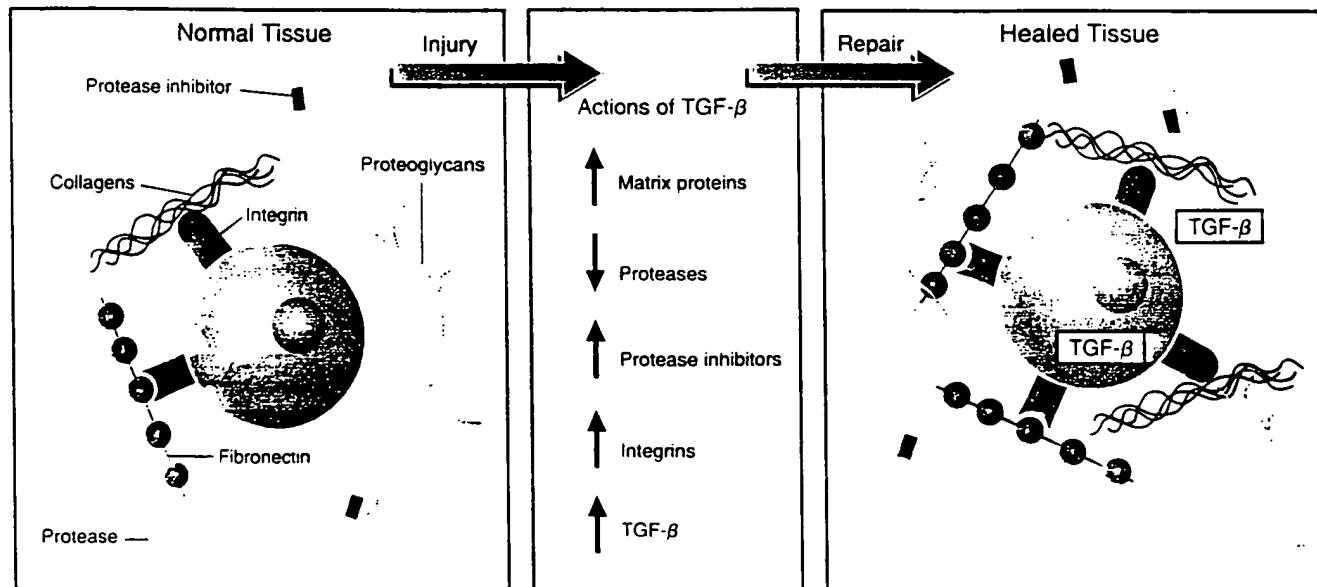


Figure 1. Actions of TGF- β in the Healing of Injured Tissue.

Platelets release TGF- β at the site of tissue injury. To repair the damage, TGF- β then induces the deposition of extracellular matrix by simultaneously stimulating the production of new matrix proteins (fibronectin, collagens, and proteoglycans), blocking matrix degradation by decreasing the synthesis of proteases and increasing the synthesis of protease inhibitors, and modulating the expression of cell-surface integrins in a manner that enhances cell-matrix interaction and matrix assembly. TGF- β also induces its own production by cells, thus amplifying its biologic effects.

TGF- β 1 causes the deposition of extracellular matrix by simultaneously stimulating cells to increase severalfold the synthesis of most matrix proteins, decrease the production of matrix-degrading proteases, increase the production of inhibitors of these proteases, and modulate the expression of integrins in a manner that increases cellular adhesion to the matrix. These comprehensive effects on the extracellular matrix reflect the diverse biologic properties of TGF- β 1 and may also be part of a negative-feedback loop that normally regulates the expression of TGF- β .²⁶ TGF- β binds to proteoglycans in the matrix or near the cell surface, and such binding may act as a signal to terminate the production of TGF- β after tissue repair is complete.

ENHANCEMENT OF WOUND HEALING BY TGF- β

Fibrosis represents a pathologic excess of the normal process of tissue repair. Excessive or sustained production of TGF- β 1 is a key molecular mediator of tissue fibrosis. The topical application of TGF- β accelerates wound healing.^{5,16} In rats, topical or limited intravenous administration of recombinant TGF- β 1 normalizes wound healing that is impaired by age or glucocorticoids.²⁷ In humans, TGF- β 2 has been used to repair retinal holes. TGF- β therefore has great promise as a therapy for poorly healing wounds.²⁸

However, the fibrogenic potential of TGF- β is revealed with repeated injections of higher doses. Two weeks of intravenous injections of TGF- β 1 produced serious systemic effects in rats, including marked fibrosis in the kidneys and liver and at the injection site.²⁹ Severe cachexia and generalized tissue fibrosis developed in mice given TGF- β 1 intraperitoneally for 10 days.³⁰

The clinical counterpart of these results may be the rapid onset of liver and lung fibrosis in patients with advanced breast cancer who receive high-dose chemotherapy in preparation for autologous bone marrow transplantation. In one study, more than 90 percent of the patients whose plasma TGF- β concentrations were 2 SD or more above the normal mean (10 ng per milliliter) had liver or lung fibrosis.³¹ The source of the elevated plasma TGF- β concentrations in these patients is unknown. Measuring TGF- β is expensive and technically cumbersome with existing bioassays, but a newly reported bioassay promises to be more sensitive and specific.³²

TGF- β IN FIBROTIC DISEASES

Table 1 lists the animal models and human disorders in which TGF- β has been implicated in the pathogenesis of fibrosis. The involvement of TGF- β in kidney, liver, and lung disease has been the most thoroughly investigated.

Kidney

The intricate architecture and filtrating function of the kidney make it particularly vulnerable to the con-

Table 1. TGF- β in Animal Models of Fibrotic Disorders and in Human Fibrotic Disorders.

ORGAN AND DISORDER	REFERENCE
Animal models	
Kidney	
Acute ATS glomerulonephritis	Okuda et al. ³³
Chronic ATS glomerulonephritis	Yamamoto et al. ³⁴
Anti-GBM glomerulonephritis	Coimbra et al. ³⁵
Habu-venom glomerulonephritis	Barnes and Abboud ³⁶
Acute and chronic puromycin-induced nephrosis	Jones et al. ³⁷
Diabetic nephropathy	Yamamoto et al. ³⁸
HIV nephropathy	Kopp et al. ³⁹
Ureteral obstruction	Kaneo et al. ⁴⁰
Angiotensin-induced nephropathy	Kagami et al. ⁴¹
Liver	
Schistosomiasis	Czaja et al. ⁴²
Carbon tetrachloride-induced hepatic fibrosis	Czaja et al. ⁴²
Lung	
Bleomycin-induced fibrosis	Westergren-Thorsson et al. ⁴³ Khalil et al. ⁴⁴
Skin	
Normal and impaired wound healing	Sporn and Roberts. ²⁸ Terrell et al. ²⁹
Arteries	
Vascular restenosis	Wolf et al. ⁴⁵
Central nervous system	
Scarring after injury	Logan et al. ⁴⁶
Other	
Acute and chronic arthritis	Brandes et al. ²³
Radiation-induced fibrosis	Barcellos-Hoff et al. ⁴⁷
Granulomas	Appleton et al. ⁴⁸
Postoperative adhesions	Williams et al. ⁴⁹
Human disease	
Kidney	
Glomerulonephritis	Yoshioka et al. ⁵⁰
Diabetic nephropathy	Yamamoto et al. ³⁸
Allograft rejection	Shihab et al. ⁵¹
HIV nephropathy	Border et al. ⁵²
Liver	
Cirrhosis	Castilla et al. ⁵³ Nagy et al. ⁵⁴
Veno-occlusive disease	
Idiopathic fibrosis	Anscher et al. ³¹ Broekelmann et al. ⁵⁵
Autoimmune fibrosis	
Skin	
Systemic sclerosis	Kulozik et al. ⁵⁷
Keloids	Peltonen et al. ⁵⁸
Hypertrophic burn scars	Ghahary et al. ⁵⁹
Eosinophilia-myalgia syndrome	Varga et al. ⁶⁰
Arteries	
Vascular restenosis	Nikol et al. ⁶¹
Central nervous system	
Intraocular fibrosis	Connor et al. ⁶²
Other	
Rheumatoid arthritis	Lafyatis et al. ⁶³
Nasal polypsis	Ohno et al. ⁶⁴

*ATS denotes antithymocyte serum. GBM glomerular basement membrane, and HIV human immunodeficiency virus.

sequences of fibrosis. A model of acute glomerulonephritis in rats has provided a unique opportunity to study the role of TGF- β 1 in fibrogenesis, because glomeruli can be rapidly isolated and studied in vitro throughout the course of disease.³³ In these rats a single injection of an antithymocyte serum injures glomerular mesangial cells. Extracellular matrix accumulates in the nephritic glomeruli, reaching a peak level in 14 days, after which the glomeruli

return to normal. The temporal pattern of *TGF-β1* gene expression and the actions of *TGF-β1* on the extracellular matrix mirror the pattern of accumulation and removal of the pathologic matrix. For example, nephritic glomeruli contain many times more *TGF-β1* mRNA than normal glomeruli, synthesize more *TGF-β1* protein, and produce much more fibronectin and proteoglycans.³³ The plasmin protease system, which is thought to have an important role in matrix degradation, is strikingly suppressed owing to a decrease in plasminogen activator and a substantial increase in the synthesis of plasminogen activator inhibitor type 1.⁶⁵ Simultaneously, the synthesis and expression of integrin receptors for fibronectin and collagen increase.⁶⁶ Platelet-derived growth factor and fibroblast growth factor also mediate some biologic events, especially cell proliferation, in these rats.⁶⁷

Three lines of evidence point to a causal relation between elevated production of *TGF-β1* and the accumulation of extracellular matrix in the model of glomerulonephritis. First, the *in vivo* events that underlie the accumulation of matrix — increased production of extracellular-matrix proteins, inhibition of protease activity, and increased integrin expression — have all been reproduced by incubation of normal glomeruli or mesangial cells as well as nonrenal cells with *TGF-β1*.^{33,65,66} Second, injecting nephritic rats with an antiserum capable of neutralizing *TGF-β1* or with a proteoglycan that binds *TGF-β1* prevented the increased production of matrix proteins by the glomeruli and blocked the accumulation of matrix.^{68,69} Third, *in vivo* transfection of the *TGF-β1* gene into normal rat kidneys led to increased production of *TGF-β1* in glomeruli and the rapid development of glomerulosclerosis.¹² Identical transfection of the gene for platelet-derived growth factor markedly stimulated the proliferation of glomerular cells, with an associated slight increase in extracellular matrix. The differences in the biologic activities of *TGF-β1* and platelet-derived growth factor, revealed in the gene-transfection experiments, are similar to earlier findings in which these cytokines were delivered *in vivo* by osmotic minipumps.¹⁵

An exciting feature of the glomerulonephritis model is the recent discovery that animals receiving a second injection of the antiserum have persistent elevations of glomerular *TGF-β1* mRNA and *TGF-β1* itself.³⁴ Myofibroblast-like cells appear in the tubulointerstitium of the kidney and strongly express *TGF-β1*. The persistent production of *TGF-β1* in the kidney leads within weeks to glomerulosclerosis and tubulointerstitial fibrosis, a picture closely resembling the histologic findings in humans with chronic glomerulonephritis.

Elevated concentrations of *TGF-β1* may also be important in the pathogenesis of glomerulosclerosis in diabetic nephropathy. Rats made diabetic with streptozocin, a drug that causes insulin deficiency, had progressively increasing concentrations of *TGF-β1*

mRNA and *TGF-β1* in their glomeruli.³⁸ The stimulus that triggers the expression of *TGF-β1* in diabetes may be hyperglycemia or an increase in the activity of the renin-angiotensin system in renal tissue. In humans, good control of blood glucose with insulin and the administration of an angiotensin-converting-enzyme inhibitor retard the development of diabetic nephropathy. In diabetic rats, insulin treatment reduced the increase in the amounts of glomerular *TGF-β1* mRNA and the extracellular-matrix proteins known to be induced by *TGF-β1*.³⁸ In cultured rat mesangial cells, both increased glucose and increased angiotensin II concentrations induced the production of *TGF-β1*, which then stimulated the synthesis of fibronectin, collagens, and proteoglycans.^{41,70} The administration of angiotensin II to rats leads to elevated amounts of glomerular *TGF-β1* mRNA and type I collagen mRNA in one week.⁴¹

The relevance of these studies to human glomerular diseases has recently been demonstrated. In kidney-biopsy specimens from patients with mesangial proliferative glomerulonephritis, a disease histologically similar to the model of glomerulonephritis described above, glomerular immunostaining for *TGF-β1* was intense, and the intensity correlated closely with the amount of mesangial matrix.⁵⁰ In the glomeruli of humans with diabetic nephropathy, *TGF-β1* protein and matrix proteins induced by *TGF-β1* were increased, as they were in the glomeruli of diabetic rats.³⁸ Glomeruli from patients with renal diseases in which fibrosis does not occur and from patients with no renal diseases were negative for *TGF-β1*. Recently, elevated amounts of *TGF-β1* protein were found in fibrotic kidneys from patients with human immunodeficiency virus-associated nephropathy and patients with chronic allograft rejection.^{51,52}

Liver

mRNA for type I collagen, the predominant matrix component in injured liver, is increased in cultured rat hepatocytes incubated with *TGF-β1*.⁴² In liver-biopsy specimens from patients with chronic liver disease, the amount of *TGF-β1* mRNA correlated closely with that of type I collagen mRNA.⁵³ *TGF-β1* mRNA concentrations in the liver are mirrored by serum concentrations of peptide fragments of type III collagen and the histologic activity of the liver disease. In biopsy specimens from patients with chronic liver disease, *TGF-β1* protein was detected by immunostaining in areas of fibrosis, but not in areas of inactive disease or in normal liver.⁵⁴ As previously discussed, elevated plasma *TGF-β* concentrations are highly predictive of the development of hepatic fibrosis (veno-occlusive disease) in the recipients of bone marrow transplants.³¹

In two models of hepatic fibrogenesis, one induced by the administration of carbon tetrachloride and the other by infection with schistosoma, increased concentrations of *TGF-β1* mRNA and *TGF-β1* in perisinu-

soidal cells paralleled the increased expression of a collagen gene and increased collagen synthesis.⁴²

Lung

In rats with pulmonary fibrosis induced by the administration of bleomycin, total lung TGF- β 1 content was several times higher than in normal rats. The increased production of TGF- β 1 preceded the synthesis of collagens, fibronectin, and proteoglycans.⁴³ The principal cellular source of TGF- β 1 was alveolar macrophages, in which increased production of TGF- β 1 could not be suppressed by high-dose corticosteroid treatment, a possible explanation for the ineffectiveness of this treatment in patients with idiopathic pulmonary fibrosis.⁴⁴

In humans with idiopathic pulmonary fibrosis, TGF- β 1 is increased in alveolar walls at the sites at which extracellular matrix has accumulated.⁵⁵ Bronchoalveolar cells obtained by lavage from patients with autoimmune diseases and lung fibrosis contained

10 times more TGF- β 1 mRNA than similar cells obtained from normal subjects or patients with asthma.⁵⁶

Fibrotic Disorders of Other Organs

TGF- β 1 and collagen are increased in tissue sections from patients with systemic sclerosis,⁵⁷ keloids,⁵⁸ and hypertrophic scars from burns.⁵⁹ TGF- β 1 has been implicated in the fibrosis associated with eosinophilia.⁶⁰ Increased amounts of TGF- β 1 are also found in the arteries of rats at the sites of balloon angioplasty and in vascular lesions associated with restenosis in humans.^{45,61}

OVERPRODUCTION OF TGF- β IN FIBROSIS

In both animals and humans, acute, limited injury is accompanied by only a transient increase in TGF- β 1, and fibrosis does not occur. With repeated injury, the increase in TGF- β 1 production is sustained, leading to the progressive deposition of extracellular matrix and tissue fibrosis.³⁴ The manner in which TGF-

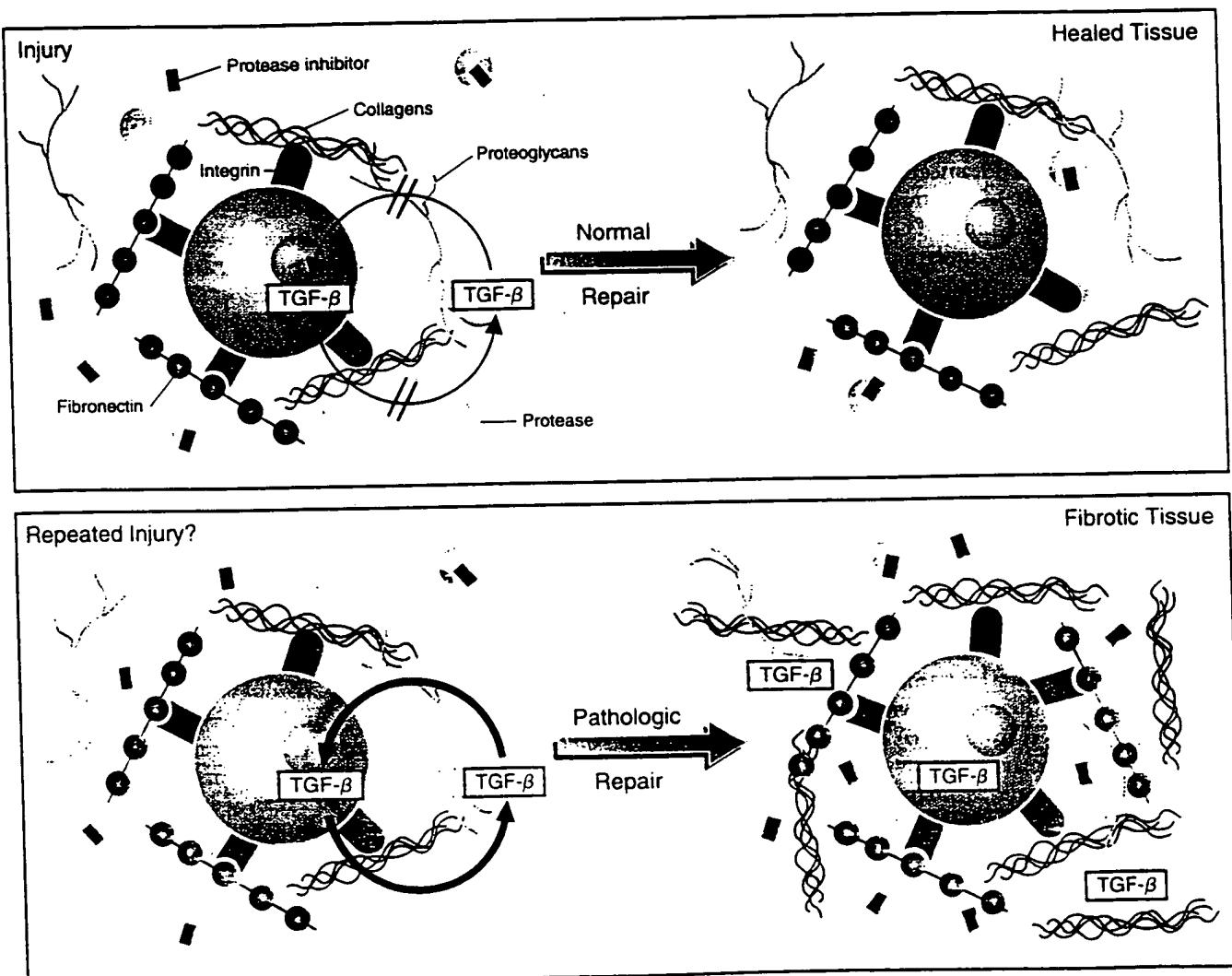


Figure 2. Overproduction of TGF- β in Fibrogenesis.

In normal tissue repair, the production of TGF- β and extracellular matrix is terminated by unknown mechanisms as the damaged tissue heals. In patients with chronic disease, repeated tissue injury, a defect in TGF- β regulation, or both lead to the continuous production of TGF- β and extracellular matrix, resulting in tissue fibrosis.

$\beta 1$ production is normally terminated is unknown. Repeated injury, with continued autoinduction of TGF- $\beta 1$, overrides the normal termination signals, creating a chronic, vicious circle of TGF- $\beta 1$ overproduction, as shown in Figure 2.

TGF- β ANTAGONISTS AS ANTIFIBROTIC AGENTS

Injecting an antiserum capable of neutralizing the activity of TGF- $\beta 1$ inhibited its action in the kidney,⁶⁸ skin,⁷¹ lung,⁷² brain,⁷³ joint,⁷³ and arterial wall.⁴⁵ In each case excessive amounts of extracellular matrix were not deposited, and tissue repair was normal. In nephritic rats, the injection of the antiserum dramatically reduced the synthesis of matrix proteins and the deposition of plasminogen activator inhibitor type 1 in the glomeruli and blocked the accumulation of mesangial matrix. The collagen content of dermal wounds treated with anti-TGF- $\beta 1$ was substantially reduced, but their tensile strength was normal and scar formation was minimal. Anti-TGF- $\beta 1$ reduced fibrous scar tissue and inflammation at the site of brain injury. In arthritic joints, anti-TGF- $\beta 1$ decreased inflammation and bone and synovial destruction. Finally, in rats with carotid-artery injury, the injection of anti-TGF- $\beta 1$ suppressed the accumulation of matrix that underlies the development of intimal hyperplasia and restenosis. These consistent therapeutic successes make clear the enormous clinical potential associated with decreasing the action of TGF- $\beta 1$ *in vivo*.

CONCLUSIONS

As the complexities of TGF- β regulation are unraveled, a number of possible therapeutic approaches for decreasing the action of the cytokine have arisen that may be more suitable than antibodies for use in humans.^{2,5} For example, soluble TGF- β type III receptors inhibit the binding of TGF- β to its membrane receptors and block its action; soluble type I and II receptors, which have higher affinities for TGF- β , may be even more potent in blocking its action.^{9,10} Similarly, the latency-associated peptide that is released in the process of TGF- β activation may be used to inhibit the action of TGF- β . Several members of the superfamily of retinoid-steroid receptors may act as post-transcriptional regulators for genes of different isoforms of TGF- β .⁷ Manipulation of this regulation may lead to decreased production of TGF- β . Using TGF- β antisense oligonucleotides is another possibility. A low dietary intake of protein decreased the expression of TGF- $\beta 1$ in rats with acute glomerulonephritis.⁷⁴ This finding may help explain the beneficial effect of low-protein diets in patients with various kidney diseases. Finally, some proteoglycans bind TGF- β .⁶⁹ The injection of one of these proteoglycans into nephritic rats was as effective as the injection of anti-TGF- $\beta 1$ in inhibiting glomerular accumulation of matrix.⁶⁹

Whether any of these approaches will yield an effective antifibrotic drug is unknown. Nevertheless, understanding that TGF- β is a key factor in fibrogenesis

offers a target for the development of new therapeutic agents for the many fibrotic conditions associated with the overproduction of TGF- β .

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